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Floral Scent Production in *Clarkia* (Onagraceae)[1]

I. Localization and Developmental Modulation of Monoterpene Emission and Linalool Synthase Activity

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The flowers of many plants emit volatile compounds as a means of attracting pollinators. We have previously shown that the strong, sweet fragrance of *Clarkia breweri* (Onagraceae), an annual plant native to California, consists of approximately 8 to 12 volatile compounds—three monoterpenes and nine benzoate derivatives (R.A. Raguso and E. Pichersky [1994] Plant Syst Evol [in press]). Here we report that the monoterpene alcohol linalool is synthesized and emitted mostly by petals but to a lesser extent also by the pistil and stamens. Two linalool oxides are produced and emitted almost exclusively by the pistil. These three monoterpenes are first discernible in mature unopened buds, and their tissue levels are highest during the first 2 to 3 d after anthesis. Levels of emission by the different floral parts throughout the life span of the flower were correlated with levels of these monoterpenes in the respective tissues, suggesting that these monoterpenes are emitted soon after their synthesis. Activity of linalool synthase, an enzyme that converts the ubiquitous C_{10} isoprenoid intermediate geranyl pyrophosphate to linalool, was highest in petals, the organ that emits most of the linalool. However, linalool synthase activity on a fresh weight basis was highest in stigma and style (i.e. the pistil). Most of the linalool produced in the pistil is apparently converted into linalool oxides. Lower levels (0.1%) of monoterpene emission and linalool synthase activity are found in the stigma of *Clarkia concinna*, a nonscented relative of *C. breweri*, suggesting that monoterpenes may have other functions in the flower in addition to attracting pollinators.

Flowers of many plants attract pollinators by producing and emitting volatile compounds. The scent emitted by such flowers is often a complex mixture of low mol wt compounds, and the relative abundances and interactions of the constituents give the flower its particular characteristic fragrance. Floral scents have been demonstrated to function as long- and short-distance attractants and nectar guides to a variety of animal pollinators (reviewed by Dobson, 1993). Moreover, insects are able to distinguish between complex floral scent mixtures (Dodson et al., 1969; Pellmyr, 1986). Discriminatory visitation based on floral scent has important implications for population structure and reproductive isolation in both temperate (Galen and Kevan, 1983; Galen, 1985; Pellmyr, 1986) and tropical plant species (Dodson et al., 1969). Thus, floral scent is of paramount importance to plant reproduction and evolution.

Several thousand compounds have been identified from various floral scents, mostly by steam distillation or headspace trapping in combination with GC-MS (Knudsen et al., 1993). Most of these compounds are either terpenoids, benzenoid compounds, or acyl lipid derivatives (Croteau and Karp, 1991). Although perfumers still survey natural sources for novel fragrance compounds (Joulain, 1987; Kaiser, 1991), this information is most often used in directing organic syntheses to imitate natural fragrances or create new combinations. Thus, although the structures of many floral scent compounds are known, very few studies have focused on the biosyntheses of these compounds in the plant cell. Few of the critical biochemical pathways have been elucidated and few, if any, of the enzymes involved in their synthesis have been identified, let alone purified and characterized.

Although the biosynthesis of terpenoids such as linalool that contribute to floral scent has not been extensively investigated, the biosynthesis of some mono- and sesquiterpene volatiles accumulated by the vegetative parts of plants (e.g. leaf glandular trichomes, stem resin ducts) has been examined in great detail (Croteau, 1986; Alonso and Croteau, 1993). All terpenoids are synthesized from successive condensation of isopentenyl PPi and its allylic isomer, dimethylallyl PPi, which are derived from the mevalonic acid pathway. Condensation of one isopentenyl PPi molecule with one dimethylallyl PPi molecule produces GPP, the ubiquitous C_{10} intermediate of the isoprenoid pathway (Gershenzon and Croteau, 1993). Monoterpenes are then synthesized from GPP by a class of enzymes termed monoterpene synthases. These operationally soluble enzymes utilize a divalent cation as the only cofactor, and many have been partially purified and characterized (Gambliel and Croteau, 1982; Lewinsohn

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Abbreviations: GPP, geranyl pyrophosphate; LIS, linalool synthase.

et al., 1992; Rajaonarivony et al., 1992; Alonso and Croteau, 1993).

We have recently begun investigating the production of scent in the flowers of *Clarkia breweri* [Gray] Green (Onagraceae), an annual plant native to California. We have shown that the strong, sweet fragrance of *C. breweri* consists of some 8 to 12 different volatiles that fall into two groups: monoterpenoids and benzenoids (Raguso and Pichersky, 1994). A major component of the scent is linalool, an acyclic monoterpene common to the floral scents of numerous other plant species (Kaiser, 1991; Knudsen et al., 1993). Two cyclic isomers of linalool oxide are also produced by *C. breweri* (Fig. 1), almost certainly by further oxidative modification of linalool. Here we describe the quantitative localization of these monoterpenes in different parts of the flower and the emission patterns of these floral organs. In addition, we establish that the activity of the enzyme LIS in the various floral organs of *C. breweri* is both developmentally and differentially regulated.

MATERIALS AND METHODS

Plant Material, Growth Conditions, Headspace Collection, GC-MS Analysis

Details of *Clarkia breweri* and *Clarkia concinna* stocks and growing conditions, dynamic headspace collection on Tenax and activated charcoal sorbents, and chemical analyses via GC-MS are as described by Raguso and Pichersky (1994). All headspace collections were performed in a Conviron (Asheville, NC) growth chamber under a 12-h light/12-h dark photoperiod. Temperature was set to 25°C during the light period and 18°C during the dark period. In all experiments, headspace collections from ambient air and from vegetative tissues were used as controls.

Time Course of Scent Production

Volatile monoterpenoid production in individual flowers of four separate plants was monitored during a 7-d period beginning on the day before anthesis and continuing until floral abscission. Headspace volatiles were collected as described by Raguso and Pichersky (1994). The collections were made at 12-h intervals, corresponding to the dark and light periods in the growth chamber. All flowers were hand-pollinated with a cotton swab on the evening of d 3 after anthesis to simulate the typical life cycle of a *C. breweri* flower.

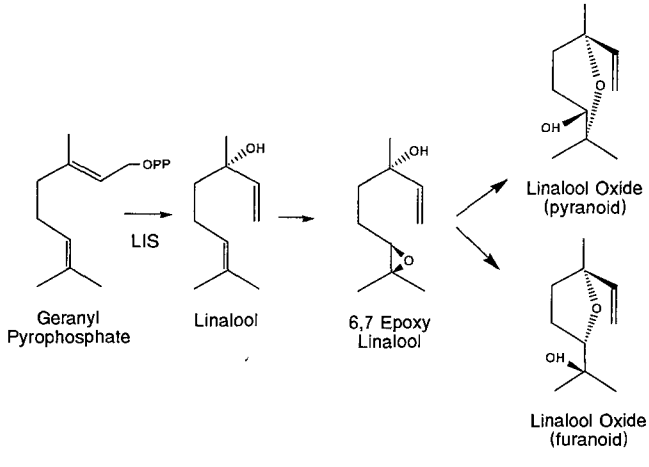

Figure 1. The proposed pathway leading from GPP to linalool and linalool oxides (see text for references).

Localization and Quantification of Monoterpene Synthesis in Floral Parts

The specific floral parts responsible for scent emission were determined and the emission levels were quantified by collection of headspace volatiles from attached, second day (hermaphroditic) intact flowers and from same-stage flowers in which floral organs had been systematically removed, to leave only petals, only anthers, or only the style. To detect all volatiles emitted by a given flower part, which could possibly emit different compounds at different times, a 24-h collection period was used. To determine diurnal variation in the emission of a given compound, headspace collections were taken during 6- and 12-h intervals.

Monoterpene Extraction

Whole flowers representing the phenological spectrum from anthesis to abscission were excised at the base of the hypanthium, weighed, and macerated with a glass rod in 1 mL of HPLC-grade hexane at ambient temperature and then stored in the dark at −20°C. Samples were concentrated to a volume of 75 μL under a flow of gaseous N_2, and 1.5 μL of a 0.3% (v/v) solution of geraniol in hexane were added as an internal standard. Four microliters of each sample were then used for GC-MS analysis.

LIS Enzyme Extraction and Assay

Enzyme Extraction

A crude protein extract was prepared by macerating flower parts in a microcentrifuge tube in the presence of ice-cold buffer (10 vol:fresh weight) containing 50 mM bis-Tris-HCl (pH 6.9), 10 mM DTT, 5 mM $Na_2S_2O_5$, 1% (w/v) PVP-40, and 10% (v/v) glycerol. The slurry was centrifuged for 10 min, and the supernatant was transferred to a new tube. For each time, extracts of three to five flowers from three different plants were combined.

Enzyme Assay and Product Analysis

LIS activity was assayed by diluting 10 μL of crude extract (1.5–3 μg of protein) into 80 μL of assay buffer (50 mM potassium Hepes [pH 7.8], 5 mM DTT, 5 mM sodium metabisulfite, 10% [v/v] glycerol, 20 mM $MgCl_2$, and 5 mM $MnCl_2$ [Lewinsohn et al., 1991]), adding 10 μL of [1-^{3}H]GPP to a final concentration of 16 μM, at 150 mCi/mol (substrate

synthesized according to the method of Croteau and Cane [1985]), and overlaying the mixture with either pentane or hexane to trap volatile metabolites. The tube was then vortexed briefly and incubated at 20°C for 1 h. Appropriate controls included the omission of crude extract, the use of boiled crude extracts, the omission of substrate and/or cations, and the substitution of other potential substrates such as (3*R*)- and (3*S*)-[1-^{3}H]linalyl PPi.

After 1 h, the tube was vortexed again and the amount of the radioactive linalool product, which partitioned into the organic phase, was determined by a liquid scintillation of an aliquot. Radio-GC analysis (Croteau and Satterwhite, 1990) of labeled products in the organic phase was also undertaken to examine the formation of geraniol (liberated from the substrate by phosphatases) and to determine whether other monoterpene synthases were present in the extracts. These radio-GC analyses showed that no floral tissue contained detectable monoterpene synthase activity other than LIS (linalool >95% of product by coincidence of radioactivity with authentic standard) and that only crude extracts from mature pollen grains contained appreciable phosphatase activity. Because of the presence of phosphatases in the pollen grains, anther tapeta were routinely excluded in the preparation of subsequent cell-free extracts.

RESULTS

Temporal Variation in Scent Emission by Intact Flowers

Under our growing conditions, the first buds became visible on the *C. breweri* plant 4 to 6 weeks after germination. The closed buds developed for approximately 12 to 18 d before the fused sepals split and reflexed, allowing the petals to open (anthesis) (Fig. 2). Anthesis in *C. breweri* typically occurs in the morning. Flowers that have been pollinated at any time after d 2 past anthesis, when stigma lobes recurve and become receptive, will begin to wilt within 24 h of pollen deposition. After 6 to 7 d, unpollinated flowers also begin to senesce and wilt. We have previously shown (Raguso and Pichersky, 1994) that three monoterpenes, linalool, furanoid linalool oxide, and pyranoid linalool oxide, are constituents of the scent of *C. breweri* flowers. To determine the amount of monoterpenes emitted at different stages of floral development, we performed time-course headspace collections at 12-h intervals, followed by GC-MS analysis. We began headspace collection with buds on the evening before they opened and ended it 6 d later, after flowers had been pollinated and then wilted and abscissed. The results are shown in Figure 3, A to C.

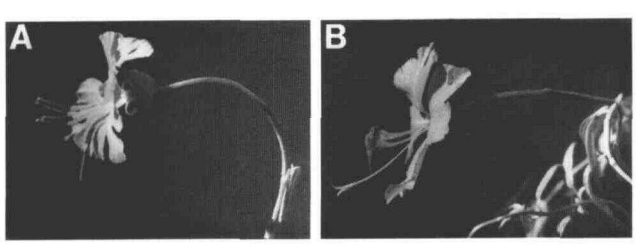

Figure 2. *C. breweri* flowers. A, A flower on d 1. Stigma has not opened yet and anthers have not dehisced. The fused sepals are visible to the right of the petals. The long pale tube is the hypanthium, terminating with the ovary, the darker tube on the right. B, A flower on d 2, showing dehisced anthers and slightly reflexed stigma.

Unopened flowers (buds) emitted no linalool or furanoid linalool oxide, but they did emit small amounts of pyranoid linalool oxide. Significant emission of all three monoterpenes began at anthesis and peaked during d 2 and afterward began a gradual decline. Monoterpene emission was still high on the evening of d 4, 24 h after hand-pollination, and then rapidly declined. Overall, the emission of linalool by the intact flowers was higher than the corresponding oxides, peaking at 8.5 μg flower^{-1} 12 h^{-1}. Emission of the pyranoid linalool oxide peaked at 5.9 μg flower^{-1} 12 h^{-1}. The furanoid linalool oxide was emitted at a much lower level, peaking at 0.4 μg flower^{-1} 12 h^{-1}. Senescence (wilting) of all flowers was observed by the morning of d 5; yet low levels of linalool and linalool oxides were emitted until abscission on the evening of d 6.

Quantitation of Monoterpenes in Flower Tissue

The amounts of monoterpenes in intact flowers at different developmental stages were determined by hexane extraction and GC-MS analysis (Fig. 3, D–F). The three monoterpenes were detectable in mature buds 2 to 3 d from anthesis (stage 5 buds, see below). Tissue levels of linalool peaked on the evening of d 2 and then declined, paralleling the pattern found for linalool emission (Fig. 3, A and D). The maximal level of linalool found in the flower tissue was approximately 10% of the amount of linalool emitted by the flower during a 12-h period. Tissue levels of linalool oxides peaked at d 3 (Fig. 3, E and F), whereas the emission of linalool oxides was highest at d 2 (Fig. 3, B and C). On d 2, levels of linalool oxides in the tissue constituted approximately 10% of the emission levels of these compounds during a 12-h period, but on d 3 this ratio increased to 20 to 60%.

Localization and Quantification of Monoterpene Emission from the Different Parts of the Flower

C. breweri Flowers

To determine the specific parts of the *C. breweri* flowers that emit monoterpenes, we performed experiments in which living flowers were modified by selectively excising floral parts, so that only one class of major floral organ (petals, stamens, pistil) remained attached to the hypanthium and sepals. We then collected headspace volatiles from these modified flowers during a 24-h period. The data obtained were used to calculate the contribution of each part to the total emission of the flower (Table I) and also the emission of each part relative to its weight (Table II). These data revealed that the majority of the linalool emission came from the petals, although substantial emission was also detected from the pistil (one-third of the amount emitted by the petals)

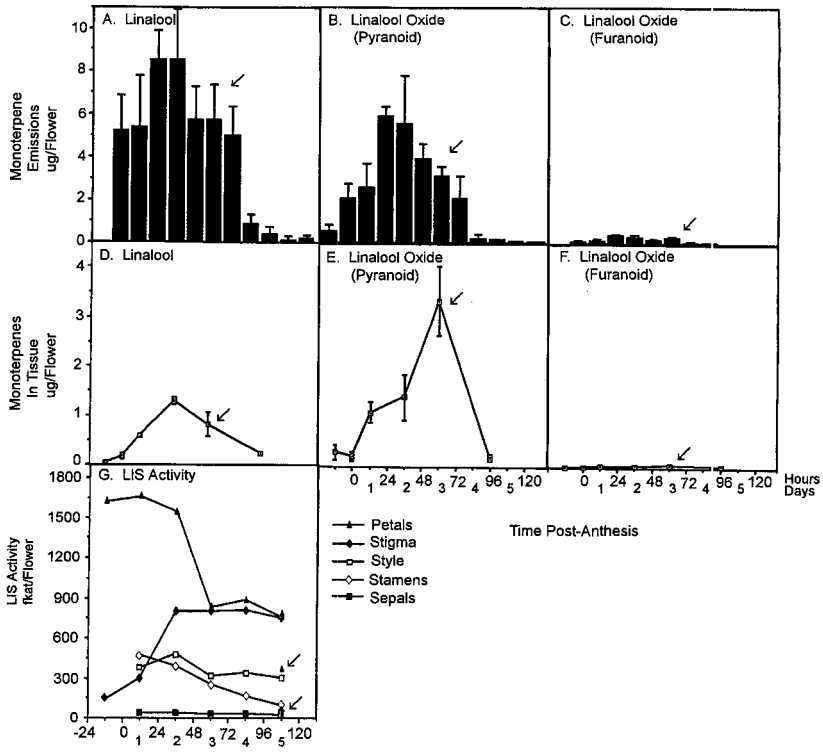

Figure 3. Emission of monoterpenes from *C. breweri* flowers and presence of these monoterpenes and LIS activity in flower tissue over time, per flower. In parts A to F, all plants were pollinated on d 3 (arrow). A, Emission of linalool, measured by headspace collection at 12-h intervals and GC-MS analysis. B, Emission of pyranoid linalool oxide analyzed as in A. C, Emission of furanoid linalool oxide analyzed as in A. D, Levels of linalool in buds and flowers determined by hexane extraction and GC-MS analysis. E, Pyranoid linalool oxide in tissue determined as described in D. F, Furanoid linalool oxide in tissue determined as described in D. G, Total LIS activity in the various floral parts. Data are presented for petals and stigma on d 5 following pollination on d 3 (arrows) or without pollination; all other data points are for nonpollinated flowers.

and some linalool was emitted by the stamens (one-tenth of the petal emission). On the other hand, pyranoid linalool oxide and furanoid linalool oxide were emitted almost exclusively by the pistil.

C. concinna Flowers

C. concinna, a close relative of *C. breweri*, has flowers that are smaller and that do not emit a detectable scent. A previous investigation revealed that *C. concinna* flowers do emit low levels of linalool and linalool oxides (0.1% of emission compared with *C. breweri*) (Raguso and Pichersky, 1994). Therefore, we examined the levels of emission of these monoterpenes in floral organs of *C. concinna* (Tables I and II). The results indicate that volatile emission occurs only in pistils.

LIS Activity in Flower Parts

LIS Activity in Flowers

To determine the site of synthesis of linalool and the temporal variation in its synthesis, we prepared crude extracts from different parts of the flowers that had been open for 1 to 6 d and assayed these extracts for activity of LIS, the enzyme that catalyzes the conversion of GPP to linalool (Fig. 1). In these experiments, we were able to dissect the flower

Table I. *Emission of monoterpenes from flower parts of C. breweri and C. concinna; 24 h collection beginning on d 2*

Species	Linalool		Pyranoid Linalool Oxide		Furanoid Linalool Oxide	
	μg	% total	μg	% total	μg	% total
C. breweri						
Stamens (n = 5)	1.31 ± 0.59	5.60	0.01 ± 0.01	0.02	0.00 ± 0.01	0.42
Pistil (n = 8)	4.78 ± 2.84	20.30	29.44 ± 5.35	129.00	0.62 ± 0.12	52.00
Petals (n = 7)	14.08 ± 4.01	60.25	0.09 ± 0.05	0.40	ND[a]	ND
Whole flowers (n = 23)	23.37 ± 1.72	100.00	22.74 ± 1.61	100.00	1.19 ± 0.13	100.00
C. concinna						
Stamens (n = 3)	ND	ND	ND	ND	ND	ND
Pistil (n = 3)	0.024 ± 0.002	100.00	0.028 ± 0.001	100.00	0.001 ± 0.001	100.00
Petals (n = 3)	ND	ND	ND	ND	ND	ND
Whole flowers (n = 3)	0.024 ± 0.002	100.00	0.03 ± 0.001	100.00	0.001 ± 0.001	100.00

[a] ND, Not detected.

Table II. *Emission of monoterpenes from flower parts of C. breweri and C. concinna; 24 h collection beginning on d 2*

Species	Linalool	Pyranoid Linalool Oxide	Furanoid Linalool Oxide
		μg/g fresh wt	
C. breweri			
Stamens (n = 5)	54.5 ± 24.4	0.2 ± 0.2	0.2 ± 0.2
Pistil (n = 8)	296.0 ± 177.6	1840.0 ± 334.5	39.0 ± 7.7
Petals (n = 7)	220.0 ± 62.7	1.4 ± 0.8	ND[a]
C. concinna			
Stamens (n = 3)	ND	ND	ND
Pistil (n = 3)	8.1 ± 0.8	9.4 ± 0.4	0.3 ± 0.2
Petals (n = 3)	ND	ND	ND

[a] ND, Not detected.

into smaller parts than was possible in the headspace collection of modified live flowers detailed above. For example, in the headspace collection it was not logistically possible to measure the contribution of the stigma separately from that of the rest of the pistil. However, when crude extracts were prepared from different parts of the flower, this limitation was overcome.

LIS activity was found in several parts of the flowers (Figs. 3G and 4). At the peak of LIS activity, at d 1 to 2, total LIS activity was similar in petals and pistil (stigma plus style) (Fig. 3G). However, stigmata possessed the highest level of LIS activity per fresh weight, followed by style tissue (with 25–35% of the specific activity found in stigma), petals (20% of stigma LIS specific activity at peak time), and stamens (Fig. 4B). None of the remaining floral parts, sepals, hypanthium, and ovaries, were found to contain any LIS activity. The vegetative parts of the plant were also devoid of LIS activity (data not shown).

Levels of LIS activity varied during the life span of the flower in concert with the levels of linalool emission and its concentration in floral tissue. Maximal LIS activity was observed during d 1 to 2. When flowers were pollinated on d 3, LIS activity decreased by 90% in the stigma and 50% in the petals within 48 h, similarly to the reduction in monoterpene emission following pollination. However, when pollination did not occur, LIS activity in the different floral organs declined only slowly from the peak levels during the next few days.

LIS Activity in Buds

Since freshly opened flowers already contain substantial enzyme activity, we investigated the level of LIS activity in buds. To carry out these experiments, it was necessary to devise a method to classify stages in bud development. Because of variation in bud size and maturation time within and among plants, it was difficult to predict the exact number of days remaining before anthesis by bud size alone. However, in contrast to bud size, we have found color changes during the development of the petals and stigma, a progression from white to dark purple, to be reliable markers for the developmental stage of the bud. Therefore, we have used these indicators to denote five stages in bud development. Each stage lasts approximately 2 to 3 d, for a total of 12 to 18 d preceding anthesis. In addition, because the small size of some floral organs within buds precluded obtaining enough material for enzyme assays, only petals and pistils were examined for LIS activity (Fig. 4A). Whereas the youngest buds (stages 1 and 2) had no detectable LIS activity, pistils (but not petals) of third-stage buds already possessed appreciable LIS activity (approximately 30% of peak level). By

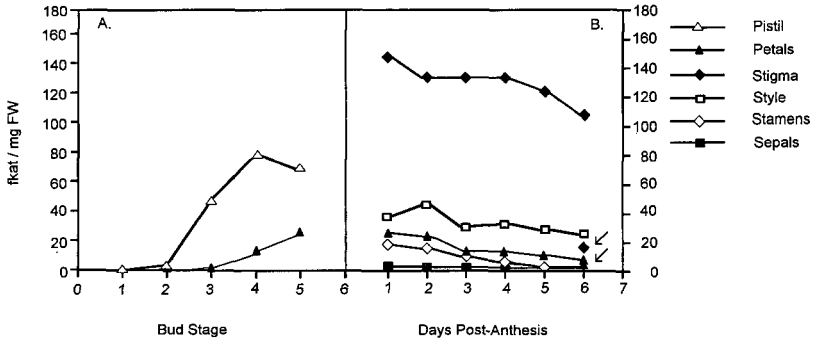

Figure 4. Levels of LIS-specific activity in different parts of buds and open flowers. Data are presented for petals and stigma on d 6 following pollination on d 3 (arrows) or without pollination. All other data points are for nonpollinated flowers. FW, Fresh weight.

stage 5, levels of LIS activity in the petals were close to or at the peak levels found in petals of open flowers.

LIS activity in flowers of C. concinna

We examined the LIS activity in extracts of *C. concinna* flower parts of d 2 flowers. Consistent with the emission data (Tables I and II), LIS activity was detected only in the stigma, which had a level of activity of 2.8 fkat/stigma (and per flower) and 4.6 fkat/g fresh weight. These levels were 0.3% of the LIS activity per stigma (0.01% per flower) and 3% per fresh weight of activity in *C. breweri* stigmata of the equivalent stage.

DISCUSSION

Temporal Variation in Scent Production by Whole Flowers and Buds

The strong, sweet, floral scent of *C. breweri* is unique in its genus and is correlated with pollination by moths, a mode of reproduction that is novel among *Clarkia* species (MacSwain et al., 1973). Emission of the monoterpene components of the scent begins as soon as the flowers are open and reaches a peak on d 2 (Fig. 3). During the life span of the flower, marked variation in monoterpene emission between the day and night periods was not observed. It is possible that temporary, but substantial, increases or decreases in emission were missed because of their short duration, but headspace collections made at 6-h intervals also failed to detect such changes, and samples did not vary by more than a factor of 1.5 (data not shown). Daily cycling in emission of linalool and other floral scent components, with a nocturnal peak in intensity coinciding with periods of moth activity, is a feature of many moth-pollinated flowers, such as *Cestrum nocturnum* (Solanaceae) (Overland, 1960; Matile and Altenburger, 1988). However, in other moth-pollinated flowers, such as *Nicotiana sylvestris* (Solanaceae), rates of linalool emission do not differ appreciably between night and day (Loughrin et al., 1990, 1991).

Localization and Quantification of Monoterpene Emission from the Different Parts of the Flower

Our data (Table I) show that two-thirds of the floral emission of linalool comes from petals, with the style contributing most of the rest. Emission of linalool per fresh weight is actually higher in the pistil than in petals (Table II), but because the total fresh weight of the pistil accounts for only 10% of the total mass of the flower and the petals account for 40% (Table III), the petal contribution is predominant. On the other hand, the pistil is responsible for nearly 100% of floral emission of the linalool oxides. When the emissions of the different floral parts are added, the results closely approximate the complete floral scent both qualitatively and quantitatively, indicating that each part is autonomous, and thus syntheses of scent components occur at the sites of emission. Previous studies (Dobson et al., 1990; Knudsen and Tollsten, 1991) also found that different parts of flowers emit different relative amounts of scent components, although absolute quantities were not reported.

The observations that relatively small pools of free monoterpenes exist within the tissues (Fig. 3, D–F) and that the changes in tissue monoterpene concentrations parallel those of monoterpene emission (Fig. 3, A–C) suggest that these compounds are not appreciably sequestered as free monoterpenes inside the cell prior to the onset of emission but are emitted as soon as they are made. The absence of both appreciable pools of scent volatiles and the biosynthetic enzymes of such compounds in mature floral tissue of *Jasminum* species prompted Watanabe et al. (1993) to discover that fragrance components were stored as nonvolatile glycosides. However, Loughrin et al. (1992) found that the level of glycosidically bound volatiles in *Nicotiana* sp. was not correlated with the emission levels of such volatiles but with the age of the flower; older, senescing flowers had higher levels of stored glycosides. We have not yet examined the presence of glycosidically bound monoterpenes in *Clarkia* flowers. However, the presence in mature *C. breweri* flowers of LIS and, likely, the other enzymes in the pathway suggests that, if such stored modified monoterpenes are present, they might not contribute to scent emission, or their contribution might not be essential.

LIS Activity in Flower Parts

The highest and second highest levels of LIS activity per fresh weight were found in the stigma and style, respectively (Fig. 4B). The pistil is the only part of the flower (excluding the ovaries) that continues to increase in size and weight after the flower opens, but its specific LIS activity does not decrease, indicating that additional LIS activity accrues there, at least during the first few days after anthesis. However, the

Table III. *Weight distribution in different parts of the C. breweri flower*

Flower Part	Total Wt	Percent of Total
	mg	
Stamens	24.0 ± 2.0	15.1
Pistil	16.0 ± 3.0	10.1
Stigma	6.0 ± 0.5	3.8
Style	10.0 ± 0.7	6.3
Petals	64.0 ± 1.0	40.2
Hypanthium and sepals	55.0 ± 7.0	34.6
Whole flower (without ovaries)	159.0 ± 8.0	100.0

petals constitute the bulk of the LIS-containing floral tissue (Table III), and they possess a similar or even higher total amount of LIS activity compared with the pistil (Fig. 3G). Each flower part that emits linalool or linalool oxides, petals, pistils, and stamens, also contains significant LIS activity, whereas flower parts that do not contain LIS activity do not contain or emit these monoterpenes. These observations lend further support to the conclusion based on the determination of emission from flowers in which a single part was left that linalool and its oxides are emitted at or near the site of synthesis and that translocation is probably not significant.

The levels of LIS activity in the different parts of the flower throughout the life span of the flower are also positively correlated with the rates of emission of linalool and the two linalool oxides. Both monoterpene emission and LIS activity in the petals, pistil, and stamens peak during the first 2 d after anthesis, and as LIS activity decreases afterward (especially after the flower has been pollinated), so do linalool and linalool oxides emissions. However, buds of later stages contain appreciable amounts of LIS activity, but they do not accumulate or emit these monoterpenes. It is likely that earlier steps in the pathway are not yet operating in the buds, either for lack of other enzymes or because of sequestration of enzymes and/or substrates in different subcellular compartments. Alternatively, monoterpenes may be synthesized in buds of later stages but may be rapidly converted to other compounds or derivatives.

The pistil displayed relatively low levels of linalool emission but substantial emission of linalool oxides (Table I). Taken together with the observation of high levels of LIS activity in stigma and style, the conclusion is inescapable that most of the linalool produced in these organs is converted into linalool oxides. Winterhalter et al. (1986) presented evidence that, in papaya tissues, the linalool oxides are synthesized from linalool via 6,7-epoxylinalool as an intermediate (Fig. 1). They further showed that the conversion of linalool to 6,7-epoxylinalool was enzymatically catalyzed, although a specific enzyme was not identified. The enzyme(s) responsible for the formation of the linalool oxides from 6,7-epoxylinalool, if such exist, have also not yet been identified.

Interestingly, a low amount of LIS activity (0.1% compared with *C. breweri* flowers and 3% on a per fresh stigma weight basis) is found in the stigma but not in any other parts of the flowers *of C. concinna*, a species that has no discernible floral scent (Raguso and Pichersky, 1994) and is not pollinated by moths (MacSwain et al., 1973). However, linalool and both linalool oxides have been detected, albeit in trace amounts, in the headspace of flowers of *C. concinna* (0.1% of that of *C. breweri*) (Table I and Raguso and Pichersky, 1994), and this emission has now been traced to the pistil (Tables I and II). The observation that LIS activity and monoterpene synthesis occur in the pistil of an essentially nonscented flower raises the possibility that linalool and/or the linalool oxides may have some physiological function in this organ that is unrelated to attracting pollinators.

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